

Fetal Tissue Sampling—Indications, Techniques, Complications, and Experience With Sampling of Fetal Skin, Liver, and Muscle

CHRISTINE CADRIN, MD, and MITCHELL S. GOLBUS, MD, *San Francisco, California*

Invasive prenatal testing has become an important way to evaluate fetuses at increased risk for hereditary disorders. In utero sampling of fetal skin, liver, and muscle may be required to diagnose before-birth disorders that cannot be diagnosed by analysis using chorionic villi or amniotic fluid. In the next few years, many of these conditions will be detected by DNA analysis, and the need for these procedures may decrease dramatically. First performed by fetoscopy, fetal tissue sampling is now most frequently done by inserting a biopsy needle under continuous ultrasonographic guidance. We describe the indications, techniques, complications, and experience with obtaining fetal skin, liver, and muscle biopsy specimens.

(Cadrin C, Golbus MS: Fetal tissue sampling—Indications, techniques, complications, and experience with sampling of fetal skin, liver, and muscle, *In Fetal Medicine [Special Issue]*. West J Med 1993; 159:269-272)

In utero sampling of fetal skin, liver, and muscle has become an important method to evaluate fetuses at increased risk for congenital abnormalities. In the past, certain genetic disorders—such as X-linked hypohidrotic ectodermal dysplasia, ornithine transcarbamylase deficiency, carbamoyl-phosphate synthetase deficiency, and nonketotic hyperglycinemia—could be diagnosed using only invasive fetal biopsy techniques. Some of these can now be diagnosed rapidly by enzyme assay or by DNA analysis using amniotic fluid cells or chorionic villi.¹⁻⁵ When meiotic recombination occurs or the results of DNA analysis are uninformative, the direct examination of fetal skin, liver, or muscle may provide the only means of prenatal diagnosis.

Fetal Skin Sampling

Genodermatoses are severe and often fatal hereditary skin disorders. Fetal skin sampling is the only method available to diagnose many of these conditions before birth. Fetal skin biopsy was first performed by fetoscopy⁶⁻⁹; it is now frequently done by percutaneously inserting a biopsy forceps under continuous ultrasonographic guidance.¹⁰ The first reports on successful prenatal diagnosis by light and electron microscopy of fetal skin biopsies concerned fetuses at risk for bullous congenital ichthyosiform erythroderma,¹¹ harlequin ichthyosis,¹² and the Herlitz syndrome, epidermolysis bullosa letalis.¹³ Over the past ten years, more than 200 cases of prenatal diagnosis by fetal skin sampling have been reported (Table 1).¹¹⁻³⁴ Between January 1979 and July

1992, fetal skin sampling was carried out for prenatal diagnosis in 23 women at the University of California, San Francisco (UCSF).³⁵ Satisfactory specimens were obtained in 22 cases. Of the 23 women, 5 had preterm deliveries. Another had a spontaneous abortion associated with chorioamnionitis two days after the sampling procedure. One woman was still pregnant at the time this was written. The prenatal diagnosis was confirmed after delivery or termination in 18 cases. In one case in which sampling was done to evaluate for ichthyosiform erythroderma, although the results of the sampling were normal, the infant was found to be mildly affected after delivery. Three women were lost to follow-up.

Indications

Genodermatoses that require fetal skin specimens for prenatal diagnosis are those disorders not diagnosable by analysis using chorionic villi or amniotic fluid components. Table 1 provides a list of genodermatoses that have been prenatally diagnosed using fetal skin specimens.

In the future, some of the conditions listed in Table 1 will be detected by DNA analysis and thereby diagnosed by either chorionic villus sampling or amniocentesis. Fetal skin sampling would be required only if the results of the DNA analysis were uninformative.

Technique

Before any fetal skin sampling procedure, the mother undergoes a preliminary ultrasonographic examination to confirm gestational age, determine fetal viability, diag-

From the Reproductive Genetics Unit, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, School of Medicine.

Reprint requests to Mitchell S. Golbus, MD, Reproductive Genetics Unit, Rm U-262, University of California Medical Center, San Francisco, CA 94143-0720.

TABLE 1.—Prenatally Detectable Genodermatoses by Fetal Skin Sampling

Disorder	Inheritance	Source
Anhidrotic ectodermal dysplasia	Autosomal recessive	Arnold et al, 1984 ¹⁴
Bullous congenital ichthyosiform erythroderma	Autosomal dominant	Golbus et al, 1980 ¹¹ ; Anton-Lamprecht, 1983 ¹⁵ ; Eady et al, 1986 ¹⁶ ; Jurkovic and Kurjak, 1989 ¹⁷
(epidermolytic hyperkeratosis)		
Nonbullous ichthyosiform erythroderma	Autosomal recessive	Perry et al, 1987 ¹⁸
Epidermolysis bullosa dystrophica (Hallopeau-Siemens).....	Autosomal recessive	Anton-Lamprecht et al, 1981 ¹⁹ ; Bauer et al, 1986 ²⁰ ; Nazzaro et al, 1989 ²¹
Epidermolysis bullosa letalis (Herlitz).....	Autosomal recessive	Rodeck et al, 1980 ¹³ ; Heagerty et al, 1987 ²² ; Bakharev et al, 1989 ²³ ; Aivazian et al, 1990 ²⁴ ; Shimizu et al, 1991 ²⁵
Harlequin ichthyosis	Autosomal recessive	Elias et al, 1980 ¹² ; Blanchet-Bardon et al, 1983 ²⁶ ; Blanchet-Bardon and Dumez, 1984 ²⁷ ; Blanchet-Bar- don et al, 1989 ²⁸ ; Suzumori and Kanzaki, 1991 ²⁹
Hypohidrotic ectodermal dysplasia*	X-linked recessive	Gilgenkrantz et al, 1989 ³⁰
Oculocutaneous albinism	Autosomal recessive	Eady et al, 1983 ³¹ ; Rosenmann et al, 1991 ³²
Sjögren-Larsson syndrome.....	Autosomal recessive	Kousseff et al, 1982 ³³ ; Trepeta et al, 1984 ³⁴

*Fetal skin sampling is done only when a DNA analysis is uninformative.

nose multiple pregnancy, diagnose fetal structural abnormalities, determine the fetal lie, and locate the placenta. Fetal skin sampling is optimally done between 17 and 20 weeks' gestation, depending on the indication.

The patient is usually premedicated with 5 to 10 mg of intravenous diazepam (Valium). The abdomen is prepared with an iodine-based solution and alcohol and draped in a sterile manner. The skin is infiltrated with a 1% lidocaine hydrochloride solution for local anesthesia. A trocar is then introduced into the uterus, and a biopsy forceps is passed through the cannula to obtain approximately 2 mm of skin, preferably the thorax, back, buttocks, or, for certain diagnoses (oculocutaneous albinism), the scalp. The entire procedure is done under continuous ultrasonographic guidance. An ultrasound examination is performed immediately after the procedure to assess fetal viability. After sampling, the specimen is placed in appropriate fixative for electron and light microscopy. The methods to evaluate fetal skin for various prenatal diagnoses have been well described.^{7,8,11-13,19,36-40}

Complications

In experienced centers, the incidence of fetal loss from fetoscopy and fetal skin biopsy is not more than 5%.⁴¹ Principal risks include spontaneous abortion, the leakage of amniotic fluid, infection, premature labor and delivery, hemorrhage from injury to the anterior abdominal wall, uterus, or placenta, maternal and fetal injuries, and cosmetic or functional injuries. To date, there have been too few fetal skin sampling procedures done by ultrasound-guided skin biopsy to draw any conclusions about safety compared with fetoscopy.

Fetal Liver Biopsy

Most inborn errors of metabolism can be diagnosed by analysis using amniotic fluid or chorionic villi; some liver enzyme abnormalities are not currently diagnosable by DNA analysis, however. Fetal liver biopsy becomes the only method available to diagnose these conditions before birth. The first reports on successful prenatal diag-

nosis by fetal liver biopsy concerned pregnancies at risk for ornithine transcarbamylase deficiency.^{42,43} Over the past ten years, 11 cases of the use of fetal liver biopsy have been reported.^{35,42-46} During this period, 16 fetal liver biopsies were done at UCSF,³⁵ and satisfactory specimens were obtained in all but 1 case. There were no spontaneous abortions or preterm deliveries. All diagnoses were confirmed after delivery or the termination of pregnancy. One woman was lost to follow-up.

Indications

Indications for fetal liver biopsy include fetuses at risk for ornithine transcarbamylase deficiency,^{42,43} carbamoyl-phosphate synthetase deficiency,^{45,46} or von Gierke glycogen storage disease, type IA.⁴⁴ Recent advances in DNA technology have identified mutations in the ornithine transcarbamylase gene^{3,4,47} and the carbamoyl-phosphate synthetase gene,⁴⁸ permitting prenatal diagnosis to be made by DNA analysis of amniotic fluid cells or chorionic villi. Fetal liver biopsy is still needed for those cases in which DNA analysis is not informative for the detected mutations.

Technique

Similar to fetal skin sampling, fetal liver biopsy requires a preliminary ultrasonographic examination. The procedure is performed optimally between 17 and 20 weeks' gestation. If needed, the patient is premedicated with 5 to 10 mg of intravenous diazepam, and a 1% lidocaine solution is given for local anesthesia. A 16.5-gauge thin-walled needle is introduced into the amniotic cavity under continuous ultrasonographic guidance. The biopsy needle is then directed below the right costal margin and into the fetal liver. Once the needle is in the liver parenchyma, a syringe is attached to aspirate fetal liver into the biopsy needle. The tissue is removed from the needle by flushing with saline solution.³⁵ The specimen is then processed for appropriate enzyme assays.⁴²⁻⁴⁶ An ultrasound examination is done immediately after the procedure to assess fetal status.

Complications

Complications that can possibly arise from fetal liver biopsy are similar to those described with fetal skin sampling. Too few fetal liver biopsies have been done to assess a meaningful complication rate for the procedure.

Fetal Muscle Biopsy

Indications

Duchenne-type muscular dystrophy is a progressive, degenerative muscle disease that is inherited as an X-linked recessive trait. Prenatal diagnosis and carrier detection for this disorder can usually be done using DNA analysis. When recombination occurs within the gene for Duchenne muscular dystrophy, DNA analysis is uninformative, or carrier status cannot be ascertained, fetal muscle biopsy with dystrophin analysis may provide the only means of prenatal diagnosis. The first successful prenatal diagnosis by dystrophin analysis of fetal muscle biopsy was performed in 1991.⁴⁹ Fetal muscle biopsy was recently used for prenatal diagnosis in three pregnant women at UCSF.⁵⁰ Two other fetal muscle biopsies were also done by the UCSF staff. In the last two cases, satisfactory specimens were obtained, and immunofluorescence studies verified the presence of normal dystrophin in both. Unfortunately, one pregnancy resulted in spontaneous abortion three weeks after the sampling, but the other resulted in the term delivery of a normal male infant.

Technique

After a preliminary ultrasonographic examination, the woman is sedated to reduce fetal movement, and the skin is infiltrated with 1% lidocaine solution. Fetal muscle is

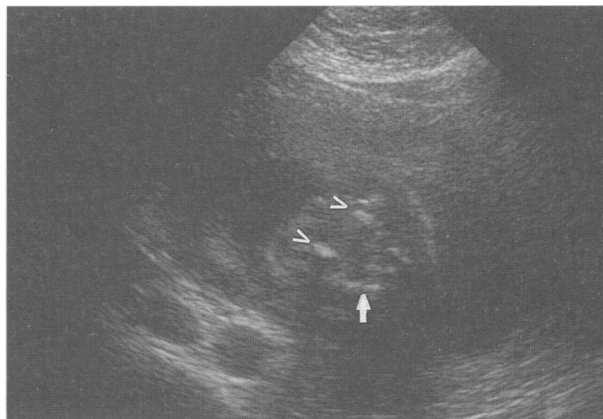


Figure 1.—The sonogram shows a fetus undergoing muscle biopsy. The closed arrow points to needle in a fetal buttock. The open arrows point to ischial tuberosities.

obtained at 16 to 22 weeks' gestation by directing a 14-gauge (Tru-Cut) biopsy needle through the maternal abdomen and obliquely into the fetal gluteal region (Figure 1). Real-time ultrasonography is used for continuous visualization. After sampling, the specimens are verified for the presence of muscle fibers, and immunoblotting or immunofluorescence is used to determine the presence or absence of dystrophin (Figure 2).⁵⁰ An ultrasound examination is done immediately after the procedure to assess fetal status.

Complications

Too few fetal muscle biopsies have been performed to assess the safety of the procedure. Possible complications include spontaneous abortion, the leakage of amniotic

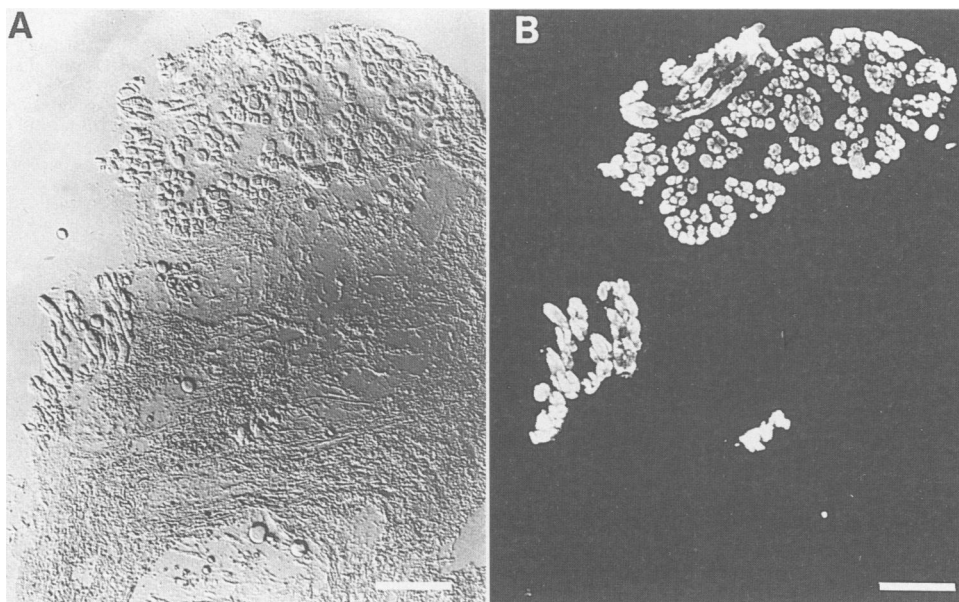


Figure 2.—The muscle fiber content is shown of a biopsy specimen using (A) Normarski optics and (B) immunofluorescence of muscle-specific myosin heavy chain protein in the same section. The muscle fibers are refractile with Normarski and stain positively for myosin. The myosin-negative tissue is epidermis and connective tissue. bar = 500 μ m

fluid, fetal or maternal hemorrhage, fetal or maternal injury, infection, prematurity, and cosmetic or functional fetal injury.

Conclusion

Over the past decade, substantial advances in invasive prenatal diagnostic techniques, such as fetal skin sampling, fetal liver biopsy, and fetal muscle biopsy, have allowed the diagnosis of congenital disorders not diagnosable using amniocentesis or chorionic villus sampling. In the next few years, many of these conditions will certainly become detectable by DNA analysis, and thereby the need for these procedures may decrease dramatically. Other invasive prenatal techniques, such as fetal kidney biopsy, might become available in the near future. Ongoing animal research on fetal hematopoietic stem cell transplantation might allow fetal liver biopsy to be used for the therapeutic application of gene transfer.⁵¹⁻⁵⁴

REFERENCES

- Zonana J, Schinzel A, Upadhyaya M, Thomas NS, Anton-Lamprecht I, Harper PS: Prenatal diagnosis of X-linked hypohidrotic ectodermal dysplasia by linkage analysis. *Am J Med Genet* 1990; 35:132-135
- Rozen R, Fox J, Fenton WA, Horwich AL, Rosenberg LE: Gene deletion and restriction fragment length polymorphisms at the human ornithine transcarbamylase locus. *Nature* 1985; 313:815-817
- Nussbaum RL, Boggs BA, Beaudet AL, Doyle S, Potter JL, O'Brien WE: New mutation and prenatal diagnosis in ornithine transcarbamylase deficiency. *Am J Hum Genet* 1986; 38:149-158
- Chadefaux B, Rabier D, Kamoun P: Prenatal diagnosis of enzymopathies of the urea cycle. *Ann Biol Clin (Paris)* 1988; 46:471-476
- Hayasaka K, Tada K, Fueri N, Aikawa J: Prenatal diagnosis of nonketotic hyperglycinemia: Enzymatic analysis of the glycine cleavage system in chorionic villi. *J Pediatr* 1990; 116:444-445
- Hogge WA, Golbus MS: Surgical management of fetal malformations. In Evans MI, Fletcher JC, Dixler AO, Shulman JD (Eds): *Fetal Diagnosis and Therapy: Science, Ethics, and the Law*. Philadelphia, Pa, Lippincott Harper, 1989, pp 395-402
- Elias S, Esterly NB: Prenatal diagnosis of hereditary skin disorders. *Clin Obstet Gynecol* 1981; 24:1069-1087
- Löfberg L, Gustavii B: 'Blind' versus direct vision technique for fetal skin sampling in cases for prenatal diagnosis. *Clin Genet* 1984; 25:37-41
- Elias S: Use of fetoscopy for the prenatal diagnosis of hereditary skin disorders. In Gedde-Dahl T, Wuepper KD (Eds): *Prenatal Diagnosis of Heritable Skin Diseases*. Basel, Switzerland, Karger, 1987, pp 1-13
- Kurjak A, Alfrevic Z, Jurkovic D: Ultrasonically guided fetal tissue biopsy. *Acta Obstet Gynecol Scand* 1987; 66:523-527
- Golbus MS, Sagebiel RW, Filly RA, Gindhart TD, Hall JG: Prenatal diagnosis of congenital bullous ichthyosiform erythroderma (epidermolytic hyperkeratosis) by fetal skin biopsy. *N Engl J Med* 1980; 302:93-95
- Elias S, Mazur M, Sabbagh R, Esterly NB, Simpson JL: Prenatal diagnosis of harlequin ichthyosis. *Clin Genet* 1980; 17:275-280
- Rodeck CH, Eady RAJ, Gosden CM: Prenatal diagnosis of epidermolysis bullosa letalis. *Lancet* 1980; 1:949-952
- Arnold ML, Rauskolb R, Anton-Lamprecht I, Schinzel A, Schmid W: Prenatal diagnosis of anhidrotic ectodermal dysplasia. *Prenat Diagn* 1984; 4:85-98
- Anton-Lamprecht I: Genetically induced abnormalities of epidermal differentiation and ultrastructure in ichthyoses and epidermolyses: Pathogenesis, heterogeneity, fetal manifestations, and prenatal diagnosis. *J Invest Derm* 1983; 81:149s-156s
- Eady RA, Gunner DB, Carbone LD, Bricarelli FD, Gosden CM, Rodeck CH: Prenatal diagnosis of bullous ichthyosiform erythroderma: Detection of tonofilament clumps in fetal epidermal and amniotic fluid cells. *J Med Genet* 1986; 23:46-51
- Jurkovic D, Kurjak A: Prenatalna dijagnostika epidermolysis bullosa hereditaria ultrazvučno vodenom biopsijom fetalne kože. *Lijec Vjesn* 1989; 111:60-63 (Eng Abstr)
- Perry TB, Holbrook KA, Hoff MS, Hamilton EF, Senikas V, Fisher C: Prenatal diagnosis of congenital non-bullous ichthyosiform erythroderma (lamellar ichthyosis). *Prenat Diagn* 1987; 7:145-155
- Anton-Lamprecht I, Rauskolb R, Jovanovic V, Kern B, Arnold ML, Schenck W: Prenatal diagnosis of epidermolysis bullosa dystrophica Hallopeau-Siemens with electron microscopy of fetal skin. *Lancet* 1981; 2:1077-1079
- Bauer EA, Ludman MD, Goldberg JD, Berkowitz RL, Holbrook KA: Antenatal diagnosis of recessive dystrophic epidermolysis bullosa: Collagenase expression in cultured fibroblasts as a biochemical marker. *J Invest Dermatol* 1986; 87:597-601
- Nazzaro V, Nicolini U, Ermacora E, Buscaglia M, Caputo R: Prenatal diagnosis of a case of Hallopeau-Siemens recessive dystrophic epidermolysis bullosa. *G Ital Dermatol Venereol* 1989; 124:1-3 (Eng Abstr)
- Heagerty AH, Eady RA, Kennedy AR, et al: Rapid prenatal diagnosis of epidermolysis bullosa letalis using GB3 monoclonal antibody. *Br J Dermatol* 1987; 117:271-275
- Bakharev VA, Karetnikova NA, Mordovtsev VN, Aivazian AA, Iantovskii IuR: Prenatal diagnosis of several hereditary skin diseases. *Akush Ginekol (Mosk)* 1989; 1:53-56
- Aivazian AA, Bakharev VA, Karetnikova NA: The prenatal diagnosis of Herlitz's borderline epidermolysis bullosa letalis. *Vestn Dermatol Venerol* 1990; 1:11-13 (Eng Abstr)
- Shimizu H, Schofield OM, Eady RA: Prenatal diagnosis of lethal junctional epidermolysis bullosa by fetal skin biopsy. *Nippon Hifuka Gakkai Zasshi [Jpn J Dermatol]* 1991; 101:539-545 (Eng Abstr)
- Blanchet-Bardon C, Dumez Y, Labbé F, et al: Prenatal diagnosis of Harlequin fetus (Letter). *Lancet* 1983; 1:132
- Blanchet-Bardon C, Dumez Y: Prenatal diagnosis of a harlequin fetus. *Semin Dermatol* 1984; 3:225-228
- Blanchet-Bardon C, Dumez Y, Labbé F, Bernheim A, Brocherion C: Diagnostic prénatal par microscopie électronique d'un fœtus Arlequin. *Ann Pathol* 1989; 3:321-325
- Suzumori K, Kanzaki T: Prenatal diagnosis of harlequin ichthyosis by fetal skin biopsy; report of 2 cases. *Prenat Diagn* 1991; 11:451-457
- Gilgenkrantz S, Blanchet-Bardon C, Nazzaro V, Formiga L, Mujica P, Alembik Y: Hypohidrotic ectodermal dysplasia—Clinical study of a family of 30 over three generations. *Hum Genet* 1989; 81:120-122
- Eady RAJ, Gunner DB, Garner A, Rodeck CH: Prenatal diagnosis of oculocutaneous albinism by electron microscopy of fetal skin. *J Invest Dermatol* 1983; 80:210-212
- Rosenmann A, Levin A, Neeman Z, Yanko L, Shenker JG, Rosenmann E: Prenatal diagnosis of albinism. *Harefuah* 1991; 120:703-704 (Eng Abstr)
- Kousseff BG, Matsuoka LY, Stenn KS, Hobbins JC, Mahoney MJ, Hashimoto K: Prenatal diagnosis of Sjögren-Larsson syndrome. *J Pediatr* 1982; 101:998-1001
- Trepeta R, Stenn KS, Mahoney MJ: Prenatal diagnosis of Sjögren-Larsson syndrome. *Semin Dermatol* 1984; 3:221-224
- Golbus MS, McGonigle KF, Goldberg JD, Filly RA, Callen PW, Anderson RL: Fetal tissue sampling—The San Francisco experience with 190 pregnancies. *West J Med* 1989; 150:423-430
- Dale BA, Perry TB, Holbrook KA, Hamilton EF, Senikas V: Biochemical examination of fetal skin biopsy specimens obtained by fetoscopy: Use of the method for analysis of keratins and flaggrin. *Prenat Diagn* 1986; 6:37-44
- Esterly NB, Elias S: Antenatal diagnosis of genodermatoses. *J Am Acad Dermatol* 1983; 8:655-662
- Löfberg L, Anton-Lamprecht I, Michaëlsson G, Gustavii B: Prenatal exclusion of Herlitz syndrome by electron microscopy of fetal skin biopsies obtained at fetoscopy. *Acta Derm Venereol (Stockh)* 1983; 63:185-189
- Löfberg L, Gustavii B: Technical difficulties in fetal skin sampling. *Acta Obstet Gynecol Scand* 1982; 61:505-507
- Anton-Lamprecht I: Prenatal diagnosis of genetic disorders of the skin by means of electron microscopy. *Hum Genet* 1981; 59:392-405
- Rodeck CH, Nicolaides KH: Fetoscopy and fetal tissue sampling. *Br Med Bull* 1983; 39:332-337
- Rodeck CH, Patrick AD, Pembrey ME, Tzannatos C, Whitfield AE: Fetal liver biopsy for prenatal diagnosis of ornithine carbamyl transferase deficiency. *Lancet* 1982; 2:297-300
- Holzgreve W, Golbus MS: Prenatal diagnosis of ornithine transcarbamylase deficiency utilizing fetal liver biopsy. *Am J Hum Genet* 1984; 36:320-328
- Golbus MS, Simpson TJ, Koresawa M, Appelman Z, Alpers CE: The prenatal determination of glucose-6-phosphatase activity by fetal liver biopsy. *Prenat Diagn* 1988; 8:401-404
- Piceni Sereni L, Bachmann C, Pfister U, Buscaglia M, Nicolini U: Prenatal diagnosis of carbamoylphosphate synthetase deficiency by fetal liver biopsy. *Prenat Diagn* 1988; 8:307-309
- Murotsuki J, Uehara S, Okamura K, et al: Prenatal diagnosis of carbamyl phosphate synthetase deficiency by fetal liver biopsy. *Nippon Sanka Fujinka Gakkai Zasshi [Acta Obstet Gynecol Japan]* 1991; 43:1613-1616 (Eng Abstr)
- Fox J, Hack AM, Fenton WA, et al: Prenatal diagnosis of ornithine transcarbamylase deficiency with use of DNA polymorphisms. *N Engl J Med* 1986; 315:1205-1208
- Haraguchi Y, Uchino T, Takiguchi M, Endo F, Mori M, Matsuda I: Cloning and sequence of a cDNA encoding human carbamyl phosphate synthetase I: Molecular analysis of hyperammonemia. *Gene* 1991; 107:335-340
- Evans MI, Greb A, Kunkel LM, et al: In utero fetal muscle biopsy for the diagnosis of Duchenne muscular dystrophy. *Am J Obstet Gynecol* 1991; 165:728-732
- Kuller JA, Hoffman EP, Fries MH, Golbus MS: Prenatal diagnosis of Duchenne muscular dystrophy by fetal muscle biopsy. *Hum Genet* 1992; 90:34-40
- Flake AW, Harrison MR, Adzick NS, Zanjan ED: Transplantation of fetal hematopoietic stem cells in utero: The creation of hematopoietic chimeras. *Science* 1986; 233:776-778
- Touraine JL, Raudrant D, Royo C, et al: In-utero transplantation of stem cells in bare lymphocyte syndrome (Letter). *Lancet* 1989; 1:1382
- Clapp DW, Dumenco LL, Hatzoglou M, Gerson SL: Fetal liver hematopoietic stem cells as a target for in utero retroviral gene transfer. *Blood* 1991; 78:1132-1139
- Moën RC: Directions in gene therapy. *Blood Cells* 1991; 17:407-416